



ELSEVIER

Biochimica et Biophysica Acta 1448 (1998) 166–170

BIOCHIMICA ET BIOPHYSICA ACTA

**BBA**

# Disorders of the calcium-sensing receptor

**R.V. Thakker \***

*MRC Molecular Endocrinology Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK*

Accepted 13 October 1998

## Abstract

The human calcium-sensing receptor (CaSR) is a 1078-amino-acid cell surface protein which is expressed in the parathyroids, thyroid cells and the kidney, and is a member of the family of G protein-coupled receptors. The CaSR allows regulation of parathyroid hormone (PTH) secretion and renal tubular calcium reabsorption in response to alterations in extracellular calcium concentrations. The human CaSR gene is located on chromosome 3q13.3–q21, and loss of function CaSR mutations have been reported in the hypercalcaemic disorders of familial benign (hypocalciuric) hypercalcaemia (FBH or FHH) and neonatal severe hyperparathyroidism (NSHPT). In addition, gain of function CaSR mutations have been observed in a novel familial syndrome of hypocalcaemia with hypercalciuria. The human CaSR gene on chromosome 3q13.3–q21 is likely to be one of several, as two other loci for FBH have been located on chromosome 19p and 19q13. Cloning and characterisation of these genes will help to further elucidate the mechanisms regulating extracellular calcium. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Hypercalcaemia; Hypocalcaemia; G-protein coupled receptor

## 1. Introduction

Calcium homeostasis by the kidneys and parathyroids is mediated by the calcium-sensing receptor (CaSR), which is located on 3q21–q24 and belongs to family C of the superfamily of G-protein coupled receptors that includes those for metabotropic glutamate, certain pheromones, and  $\gamma$ -aminobutyric acid (GABA<sub>B</sub>) [1,2]. Inactivating CaSR mutations result in familial benign (hypocalciuric) hypercalcaemia (FBH or FHH), whilst activating mutations result in hypocalcaemic hypercalciuria [3–8].

## 2. Disorders due to loss of CaSR function

Two hypercalcaemic disorders due to mutations of the CaSR have been reported [4,5,9–12]; these are familial benign hypercalcaemia (FBH), which is also referred to as familial hypocalciuric hypercalcaemia (FHH), and neonatal severe hyperparathyroidism (NSHPT). FBH is an autosomal dominant disorder characterised by lifelong and generally asymptomatic hypercalcaemia [13,14]. Other biochemical features include mild hypermagnesaemia, normal or mildly elevated serum PTH concentrations and an inappropriately low urinary calcium excretion (calcium clearance to creatinine clearance ratio < 0.01) [13–16]. The disorder is considered to be benign as patients with FBH are usually asymptomatic. However, there is an increased prevalence of

\* Fax: +44-181-383-8306; E-mail: [rthakker@rpms.ac.uk](mailto:rthakker@rpms.ac.uk)

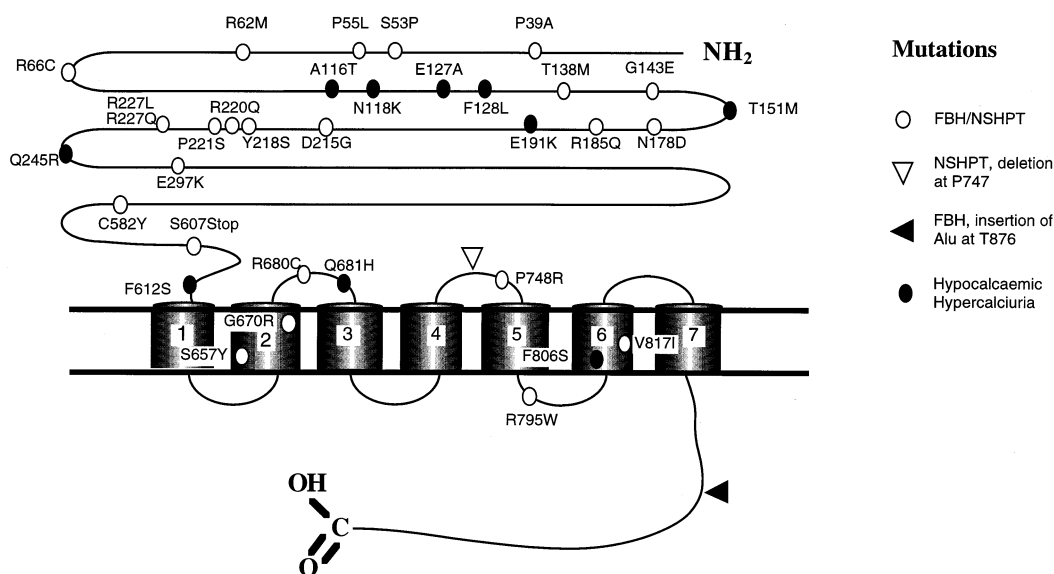


Fig. 1. Location of calcium-sensing receptor mutations found in FBH, NSHPT and hypocalcaemic hypercalciuric kindreds. A schematic representation of the calcium-sensing receptor, which consists of a large extracellular domain, seven transmembrane domains and an intracellular carboxy-terminal domain, is shown together with 36 different mutations. Each missense mutation is shown in the single letter amino acid code: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. The nonsense mutation found at codon 607 is shown as S607stop.

chondrocalcinosis with advancing age and occasional cases of acute pancreatitis have been reported [13,15–17]. An association of a progressive elevation in serum PTH concentration, hypophosphataemia and osteomalacia with FBH in a five-generation FBH kindred from Oklahoma (FBH<sub>Ok</sub>) has also been documented [18]. In contrast, NSHPT is a life-threatening disorder characterised by severe neonatal hypercalcaemia, failure to thrive, bony undermineralisation, multiple fractures and rib cage deformity [16]. NSHPT was recognised amongst some children born to consanguineous FBH parents and was thus considered to be the homozygous phenotype of FBH [19–21].

Investigations of FBH had revealed an abnormality in calcium sensing that was associated with an altered 'set point' for the regulation of PTH by ionic calcium [22,23]. Expression cloning studies, in which RNA from bovine parathyroid glands was injected into *Xenopus* oocytes, were successful in isolating a bovine parathyroid calcium-sensing receptor (BoPCaR1) [24]. The BoPCaR1 gene encodes a protein of 1085 amino acids with three structural domains that consisted of: a large N-terminal domain of 613 amino acids containing nine potential N-linked

glycosylation sites consistent with an extracellular location; a central core of 250 amino acids, containing seven hydrophobic helices characteristic of the G protein-coupled receptor superfamily; and a predominantly hydrophilic 222-amino-acid C-terminus predicted to be cytoplasmic. The BoPCaR1 has homology to the brain metabotropic glutamate receptors (mGluRs), having 29% amino acid identity to the rat mGluR1 protein, and the bovine, human and rat CaSRs and the mGluRs probably share a common framework of secondary structure with 20 conserved cysteine residues [25]. The human homologue of the BoPCaR1 gene, which shares 93% overall amino acid identity with BoPCaR1 and the rat CaSR, was mapped to chromosome 3q13–21 [4,26–29]. The CaSR functions through the activation of phospholipase C via an as yet uncharacterised, but pertussis toxin-insensitive, G protein in the parathyroid cells and allows the regulation of PTH secretion [24]. Interestingly, the rat CaSR is also expressed in the thyroid C-cells, the renal medullary thick ascending limb, cerebral arteries, brain nerve terminals, hypothalamus and pituitary [26–28].

Mutational analyses of the human CaSR have revealed different mutations, that result in a loss of

function of the CaSR in patients with FBH and NSHPT [4,6,9–12] (Fig. 1). Many of these mutations cluster around the aspartate and glutamate rich regions (codons 39–300) within the extracellular domain of the receptor [4,6,9,11,12], and this has been proposed to contain low-affinity calcium-binding sites, based on similarities to that of calsequestrin, in which the ligand-binding pockets also contain negatively charged amino acid residues [24]. Approximately two-thirds of the FBH kindreds investigated have been found to have unique heterozygous mutations of the CaSR [4,6,9–12]. Expression studies of the FBH associated CaSR mutations have demonstrated a loss of CaSR function whereby there is an increase in the calcium ion-dependent set-point for PTH release from the parathyroid cell [4,12]. NSHPT occurring in the offspring of consanguineous FBH families has been shown to be due to homozygous CaSR mutations [4,9,10,30]. However, patients with sporadic NSHPT have been reported to be associated with *de novo* heterozygous CaSR mutations [6], thereby suggesting that factors other than mutant gene dosage [30]; for example, the degree of set-point abnormality, the bony sensitivity to PTH and the maternal extracellular calcium concentration may also all play a role in the phenotypic expression of a CaSR mutation in the neonate. The remaining one-third of FBH families in whom a mutation within the coding region of the CaSR has not been demonstrated may either have an abnormality in the promoter of the gene or a mutation at one of the two other FBH loci that have been revealed by family linkage studies. These FBH loci are located on chromosome 19p and 19q13.

### 3. Disorders due to gain of CaSR function

CaSR mutations that result in a loss of function are associated with familial hypocalciuric hypercalcaemia (FHH) [4,5,9–12], and it was speculated that CaSR mutations that resulted in a gain of function may lead to hypocalcaemia with hypercalciuria. Investigation of kindreds with autosomal dominant forms of hypocalcaemia have identified such CaSR mutations [7,31–34]. Affected individuals from such families generally have normal serum intact PTH concentrations and hypomagnesaemia. However,

treatment with vitamin D or its active metabolites to correct the hypocalcaemia has resulted in marked hypercalciuria, nephrocalcinosis, nephrolithiasis and renal impairment, which was partially reversible after cessation of the vitamin D treatment. All of the 10 CaSR mutations resulting in such a functional gain have been missense mutations, and eight of these are located within the extracellular domain [7,31–34] (Fig. 1). This suggests that these mutations of the extracellular domain may increase the affinity of the receptor for calcium binding. Thus, it is important to identify and avoid vitamin D treatment in such patients and families whose hypocalcaemia is due to a CaSR abnormality and not hypoparathyroidism.

### 4. Conclusion

The identification of the extracellular calcium-sensing receptor (CaSR) has helped to define a key component in the control of the calcium homeostasis. In addition, loss of function of the CaSR has been shown to be associated with hypercalcaemic disorders of FHH and NSHPT, whilst gain of CaSR function is associated with a novel familial syndrome of hypocalcaemia with hypercalciuria.

### Acknowledgements

I am grateful to the Medical Research Council (MRC) UK, for support and to Mrs Sheila Kingsley for typing the manuscript.

### References

- [1] E.M. Brown, Mutations in the calcium-sensing receptor and their clinical implications, *Hormone Res.* 48 (1997) 199–208.
- [2] S.C. Hebert, E.M. Brown, H.W. Harris, Role of the  $\text{Ca}^{2+}$  sensing receptor in divalent mineral ion homeostasis, *J. Exp. Biol.* 200 (1997) 295–302.
- [3] Y.H. Chou, E.M. Brown, T. Levi, G. Crowe, A.B. Atkinson, H.J. Arnqvist, G. Toss, G.E. Fuleihan, J.G. Seidman, C.E. Seidman, The gene responsible for familial hypocalciuric hypercalcaemia maps to chromosome 3q in four unrelated families, *Nat. Genet.* 1 (1992) 295–300.
- [4] M.R. Pollak, E.M. Brown, Y.H. Chou, S.C. Hebert, S.J.

- Marx, B. Steinman, T. Levi, C.E. Seidman, J.G. Seidman, Mutations in the human  $\text{Ca}^{2+}$  sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism, *Cell* 75 (1993) 1297–1303.
- [5] M.R. Pollak, E.M. Brown, H.L. Estep, P.N. McLaine, O. Kifor, J. Park, S.C. Hebert, C.E. Seidman, J.G. Seidman, Autosomal dominant hypocalcaemia caused by a  $\text{Ca}^{2+}$ -sensing receptor gene mutation, *Nat. Genet.* 8 (1994) 303–307.
- [6] S.H. Pearce, D. Trump, C. Wooding, G.M. Besser, S.L. Chew, D.B. Grant, D.A. Heath, I.A. Hughes, C.R. Paterson, M.P. Whyte, R.V. Thakker, Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidism, *J. Clin. Invest.* 96 (1995) 2683–2692.
- [7] S.H. Pearce, C. Williamson, O. Kifor, M. Bai, M.G. Coulthard, M. Davies, N. Lewis-Barned, D. McCredie, H. Powell, P. Kendall-Taylor, E.M. Brown, R.V. Thakker, A familial syndrome of hypocalcaemia with hypercalciuria due to mutations in the calcium-sensing receptor, *N. Engl. J. Med.* 335 (1996) 1115–1122.
- [8] H. Heath III, C.E. Jackson, B. Otterud, M.F. Leppert, Genetic linkage analysis in familial benign (hypocalciuric) hypercalcemia: evidence for locus heterogeneity, *Am. J. Hum. Genet.* 53 (1993) 93–200.
- [9] Y.H. Chou, M.R. Pollak, M.L. Brandi, G. Toss, H. Arnqvist, A.B. Atkinson, S.E. Papapoulos, S. Marx, E.M. Brown, J.G. Seidman, C.E. Seidman, Mutations in the human  $\text{Ca}^{2+}$ -sensing receptor gene that cause familial hypocalciuric hypercalcaemia, *Am. J. Hum. Genet.* 56 (1995) 1075–1079.
- [10] N. Janicic, Z. Pausova, D.E.C. Cole, G.N. Hendy, Insertion of an Alu sequence in the  $\text{Ca}^{2+}$ -sensing receptor gene in familial hypocalciuric hypercalcaemia and neonatal severe hyperparathyroidism, *Am. J. Hum. Genet.* 56 (1995) 880–886.
- [11] K. Aida, S. Koishi, M. Inoue, M. Nakazato, M. Tawata, T. Onaya, Familial hypocalciuric hypercalcemia associated with mutation in the human  $\text{Ca}^{2+}$ -sensing receptor gene, *J. Clin. Endocrinol. Metab.* 80 (1995) 2594–2598.
- [12] H. Heath III, S. Odelberg, C.E. Jackson, B.T. Teh, N. Hayward, C. Larsson, N.R.M. Buist, K.J. Krapcho, B.C. Hung, I.V. Capuano, J.E. Garrett, M.F. Leppert, Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalciuric hypercalcaemia suggest receptor functional domains, *J. Clin. Endocrinol. Metab.* 81 (1996) 1312–1317.
- [13] S.J. Marx, M.F. Attie, M.A. Levine, A.M. Spiegel, R.W. Downs Jr., R.D. Lasker, The hypocalciuric or benign variant of familial hypercalcaemia. Clinical and biochemical features of fifteen families, *Medicine (Baltimore)* 60 (1981) 397–412.
- [14] C.R. Paterson, A. Gunn, Familial benign hypercalcaemia, *Lancet* 2 (1981) 61–63.
- [15] W.M. Law, H. Heath, Familial benign hypercalcaemia (hypocalciuric hypercalcaemia). Clinical and pathogenetic study of 21 families, *Ann. Intern. Med.* 102 (1985) 511–519.
- [16] H. Heath III, Familial benign (hypocalciuric) hypercalcaemia, a troublesome mimic of mild primary hyperparathyroidism, *Endocrinol. Metab. Clin. N. Am.* 18 (1989) 723–740.
- [17] M. Davies, P.S. Klimiuk, P.H. Adams, G.A. Lumb, D.M. Large, D.C. Anderson, Familial hypocalciuric hypercalcaemia and acute pancreatitis, *Br. Med. J.* 282 (1981) 1023–1025.
- [18] C.T. McMurtry, F.W. Schranck, D.A. Walkenhorst, W.A. Murphy, D.B. Kocher, S.L. Teitelbaum, R.C. Rupich, M.P. Whyte, Significant developmental elevation in serum parathyroid hormone levels in a large kindred with familial benign (hypocalciuric) hypercalcemia, *Am. J. Med.* 93 (1992) 247–258.
- [19] D.A. Hillman, C.R. Scriver, S. Pedvis, I. Shragovitch, Neonatal familial primary hyperparathyroidism, *N. Engl. J. Med.* 270 (1964) 483–490.
- [20] S.J. Marx, M.F. Attie, A.M. Spiegel, M.A. Levine, R.D. Lasker, M. Fox, An association between neonatal severe primary hyperparathyroidism and familial hypocalciuric hypercalcemia in three kindreds, *N. Engl. J. Med.* 306 (1982) 257–264.
- [21] B. Steinmann, H.E. Gnehm, V.H. Rao, H.P. Kind, A. Prader, Neonatal severe primary hyperparathyroidism and alkaptonuria in a boy born to related parents with familial hypocalciuric hypercalcaemia, *Helv. Paediatr. Acta* 39 (1984) 171–186.
- [22] J. Auwerx, M. Demedts, R. Bouillon, Altered parathyroid set point to calcium in familial hypocalciuric hypercalcemia, *Acta Endocrinol.* 106 (1984) 215–218.
- [23] S. Khosla, P.R. Ebeling, A.F. Firek, M.M. Burritt, P.C. Kao, H. Heath, Calcium infusion suggests a ‘set point’ abnormality of parathyroid gland function in familial benign hypercalcemia and more complex disturbances in primary hyperparathyroidism, *J. Clin. Endocrinol. Metab.* 76 (1993) 715–720.
- [24] E.M. Brown, G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M.A. Hediger, J. Lytton, S.C. Herbert, Cloning and characterization of an extracellular  $\text{Ca}^{2+}$ -sensing receptor from bovine parathyroid, *Nature* 366 (1993) 575–580.
- [25] K.H. Houamed, J.L. Kuijper, T.L. Gilbert, B.A. Haldeman, P.J. O’Hara, E.R. Mulvihill, W. Almers, F.S. Hagen, Cloning, expression and gene structure of a G-protein coupled glutamate receptor from rat brain, *Science* 252 (1991) 1318–1321.
- [26] D. Riccardi, J. Park, W.S. Lee, G. Gamba, E.M. Brown, S.C. Herbert, Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor, *Proc. Natl. Acad. Sci. U.S.A.* 92 (1995) 131–135.
- [27] M. Ruat, M.E. Molliver, A.M. Snowman, S.H. Snyder, Calcium sensing receptor: Molecular cloning in rat and localisation to nerve terminals, *Proc. Natl. Acad. Sci. U.S.A.* 92 (1995) 3161–3165.
- [28] J.E. Garrett, I.V. Capuano, L.G. Hammerland, B.C. Hung, E.M. Brown, S.C. Hebert, E.F. Nemeth, F. Fuller, Molec-

- ular cloning and functional expression of human parathyroid calcium receptor cDNAs, *J. Biol. Chem.* 270 (1995) 12919–12925.
- [29] N. Janicic, E. Soliman, Z. Pausova, M.F. Seldin, M. Riviere, J. Szpirer, C. Szpirer, G.N. Hendy, Mapping of the calcium-sensing receptor gene (CaSR) to human chromosome 3q13.3–21 by fluorescent in situ hybridization, and localization to rat chromosome 11 and mouse chromosome 16, *Mammal. Genome* 6 (1995) 798–801.
- [30] M.R. Pollak, Y.H.W. Chou, S.J. Marx, B. Steinmann, D.E. Cole, M.L. Brandi, S.E. Papapoulos, F.H. Menko, G.N. Hendy, E.M. Brown, C.E. Seidman, J.G. Seidman, Familial hypocalciuric hypercalcaemia and neonatal severe hyperparathyroidism. Effects of mutant gene dosage on phenotype, *J. Clin. Invest.* 93 (1994) 1108–1112.
- [31] M.R. Pollak, E.M. Brown, H.L. Estep, P.N. McLaine, O. Kifor, J. Park, S.C. Hebert, C.E. Seidman, J.G. Seidman, Autosomal dominant hypocalcaemia caused by a calcium-sensing receptor gene mutation, *Nat. Genet.* 8 (1994) 303–307.
- [32] D.N. Finegold, M.M. Armitage, M. Galiani, T.C. Matise, M.R. Pandian, Y.M. Perry, R. Deka, R.E. Ferrell, Preliminary localisation of a gene for autosomal dominant hypoparathyroidism to chromosome 3q13, *Pediatr. Res.* 36 (1994) 414–417.
- [33] Y.M. Perry, D.N. Finegold, M.M. Armitage, R.E. Ferrell, A missense mutation in the Ca-sensing receptor causes familial autosomal dominant hypoparathyroidism, *Am. J. Hum. Genet.* 55 (1994) A17.
- [34] J. Baron, K.K. Winer, J.A. Yanovski, A.W. Cunningham, L. Laue, D. Zimmerman, G.B. Cutler Jr., Mutations in the  $\text{Ca}^{2+}$ -sensing receptor gene cause autosomal dominant and sporadic hypoparathyroidism, *Hum. Mol. Genet.* 5 (1996) 601–606.